

Application No. 09/914,552  
Supplemental Amendment dated September 11, 2003  
Reply to Office Action dated February 26, 2003

### **IN THE CLAIMS**

This listing of claims will replace all prior versions, and listings, of claims in the application:

#### **Listing of claims:**

1. (currently amended) A method for assaying a specific component in a lipoprotein fraction in a serum by an enzymatic reaction, which comprises introducing a first reagent comprising an ion strength increasing compound, and a nonionic surfactant, and a second reagent comprising a first enzyme reacting the cholesterol in the high-density lipoprotein and a second enzyme comprising cholesterol dehydrogenase or cholesterol oxidase, or both, for enabling an enzymatic reaction preferentially with respect to an object component in the specific lipoprotein fraction without forming complexes nor aggregates, thereby specifically assaying the component.

2-26 (cancelled)

27. (previously presented) A reagent kit for detecting a cholesterol in a high-density lipoprotein, comprising a first reagent and a second reagent, wherein said first reagent comprises an ion strength increasing compound and a nonionic surfactant, and said second reagent comprises a first enzyme reacting the cholesterol in the high-density lipoprotein and a second enzyme comprising cholesterol dehydrogenase or cholesterol oxidase, or both.

28. (previously presented) The reagent kit of claim 27, wherein the ion strength increasing compound is hydrazine, hydrazine salt, hydrazine hydrate, hydrazine solvate, NaCl, urea, guanidine, or semicarbazide.

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29. (previously presented) The reagent kit of claim 27, wherein the ion strength increasing compound is hydrazine.

30. (previously presented) The reagent kit of claim 29, wherein the first reagent comprises the hydrazine of 30mM or more.

31. (previously presented) The reagent kit of claim 27, wherein the nonionic surfactant has a HLB value of 16 or more.

32. (previously presented) The reagent kit of claim 27, wherein the first enzyme is lipoprotein lipase or cholesterol esterase.

33. (previously presented) The reagent kit of claim 32, wherein the first enzyme is derived from *Chromobacterium viscosum*.

34. (previously presented) The reagent kit of claim 27, wherein the second enzyme is cholesterol dehydrogenase, and the first reagent comprises  $\beta$ -nicotinamide adenine dinucleotide of the oxidized type, thionicotinamide adenine dinucleotide of the oxidized type,  $\beta$ -nicotinamide adenine dinucleotide phosphate of the oxidized type or thionicotinamide adenine dinucleotide phosphate of the oxidized type.

35. (currently amended) A reagent kit for detecting a cholesterol in a low-density lipoprotein, comprising a first reagent and a second reagent, wherein said first reagent comprises an ion strength increasing compound, a first nonionic surfactant, a first enzyme reacting a cholesterol in a high-density lipoprotein and a second enzyme selected from cholesterol dehydrogenase or cholesterol oxidase, or both and the second reagent further comprising a second nonionic surfactant.

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36. (previously presented) The reagent kit of claim 35, wherein the second reagent comprises a third enzyme reacting the cholesterol in the low-density lipoprotein.

37. (previously presented) The reagent kit of claim 36, wherein the third enzyme is lipoprotein lipase or cholesterol esterase.

38. (previously presented) The reagent kit of claim 37, wherein the third enzyme is derived from *Pseudomonas*.

39. (previously presented) The reagent kit of claim 35, wherein the second nonionic surfactant has a HLB value of 11 to 13.

40. (previously presented) The reagent kit of claim 35, wherein the ion strength increasing compound is hydrazine, hydrazine salt, hydrazine hydrate, hydrazine solvate, NaCl, urea, guanidine, or semicarbazide, or combinations thereof.

41. (previously presented) The reagent kit of claim 35, wherein the ion strength increasing compound is hydrazine.

42. (previously presented) The reagent kit of claim 41, wherein the first reagent comprises the hydrazine of 30mM or more.

43. (previously presented) The reagent kit of claim 35, wherein the first nonionic surfactant has a HLB value of 16 or more.

44. (previously presented) The reagent kit of claim 35, wherein the first enzyme is lipoprotein lipase or cholesterol esterase, or both.

45. (previously presented) The reagent kit of claim 44, wherein the first enzyme is derived from *Chromobacterium viscosum*.

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46. (previously presented) The reagent kit of claim 35, wherein the second enzyme is cholesterol dehydrogenase, and

the first reagent comprises  $\beta$ -nicotinamide adenine dinucleotide of the oxide type, thionicotinamide adenine dinucleotide of the oxide type,  $\beta$ -nicotinamide adenine dinucleotide phosphate of the oxide type, or thionicotinamide adenine dinucleotide phosphate of the oxide type, or combinations thereof.

47. (previously presented) A method of assaying cholesterol, comprising:  
providing the kit of claim 27; and

utilizing the kit to assay a lipoprotein fraction of a patient.

48. (previously presented) A method of assaying cholesterol, comprising:  
providing the kit of claim 35; and

utilizing the kit to assay a lipoprotein fraction of a patient.

49. (new) A method for assaying a specific component in a lipoprotein fraction in a serum by an enzymatic reaction, which comprises introducing an ion strength increasing compound, a first nonionic surfactant, a first enzyme reacting the cholesterol in the high-density lipoprotein, a second enzyme selected from cholesterol dehydrogenase or cholesterol oxidase, or both and a second nonionic surfactant, for enabling an enzymatic reaction preferentially with respect to an object component in the specific lipoprotein fraction without forming complexes nor aggregates, thereby specifically assaying the component.